Review of "Diazinon and Pesticide-Related Toxicity in Bay Area Urban Creeks: Water Quality Attainment Strategy and Total Maximum Daily Load (TMDL) & Proposed Basin Plan Amendment and Staff Report"

Prepared by:
Allan S. Felsot
Professor, Entomology & Environmental Toxicology
Washington State University (afelsot@tricity.wsu.edu)

Specific Comments Related to the Technical Issues Subject to Peer Review

Introductory Comments:

The Proposed Basin Plan Amendment relies on both narrative and numerical water quality standards to bring urban storm drain discharges into compliance with the TMDL requirements of section 303(d) of the Clean Water Act. To paraphrase the narrative standard, if storm water discharges cause toxicity in established EPA recommended WET (whole effluent toxicity) test protocols for water and sediment (acute or chronic), then water quality is deemed impaired. The narrative standard will be reached through numeric targets. One numerical standard sets a specific concentration level for a single pesticide (diazinon) of 100 ng/L. The second numerical standard relies on the toxic units (TC) concept determined by conducting water column or sediment bioassays, presumably during routine collection of water/sediment samples for compliance with NPDES permitting.

The Staff Report has presented the results of past aquatic toxicity testing on waters collected in the urban Bay region to justify the development and implementation of a TMDL applicable to the water column. The report refers to some unpublished research that urban sediments may be affected by pyrethroid toxicity, but data are not presented. However, in recognition of the EPA influenced phase-out of diazinon for urban uses and final cancellation of registrations, the Staff has been progressive in planning for substitute pesticide use (mainly pyrethroids) and proposing a TMDL that anticipates water quality impairment as defined by the narrative objectives.

Comment on Numeric Targets:

The Numerical Standard for Diazinon. The Proposed Basin Plan Amendment explicitly sets a TMDL for diazinon of 100 ng/L. According to a thorough analysis conducted by the California Department of Fish and Game (Siepmann and Finlayson 2002), a probabilistically based final acute value (FAV) and final chronic value (FCV) for diazinon could be estimated as 160 ng/L and 50 ng/L, respectively. An uncertainty factor of 2 was applied to the FAV to derive a Critical Maximum Concentration (CMC) of 80 ng/L that is effectively an ambient water quality criteria for short term exposure (defined as a one-hour average exposure). The FCV becomes the CCC (Critical Continuous Concentration) and is defined as the average 4-day exposure concentration. Thus, the proposed 100 ng/L concentration is somewhat higher than the probabilistically based water quality guideline calculated by the California Department of Fish and Wildlife. However, given the inherent error associated with quantification of residues near the practical limit of detection, 100 ng/L and 80 or 50 ng/L may not be practically different if the LOD and LOQ are defined using a statistical approach with an error of p=0.001 to control both false positive and false negative detections.

The choice of 100 ng/L raises the question of whether the standard is protective enough. The Proposed Basin Plan Amendment contains sufficient data to conclude that recent water samples show decreasing trends in diazinon concentration and also decreasing probability of toxicity based on WET testing. The peer-reviewed scientific journal literature also supports the protectiveness of the 100 ng/L criterion. For example, many aquatic toxicology studies thus far have concurred that Cladocerans (specifically the species *Ceriodaphnia dubia*) are the most susceptible organisms to acute toxicity by diazinon. Bioassays of *Ceriodaphnia* in water collected from the San Joaquin River (SJR) at Vernalis, an agriculturally influenced area with historically high diazinon uses and proven dormant season runoff, showed that toxicity did not occur when diazinon concentrations were below ~150 ng/L (Kuivila and Foe 1995). These observations were supported by later observations for lack of toxicity in the same sampling location during February of 1994 and 1995 (Werner et al. 2000).

Although the Basin Plan Amendment negatively criticizes EPA regulatory mechanisms under FIFRA as not being "protective" enough for urban creeks of the Bay area, a more critical analysis of EPA's ecological risk assessment for diazinon as part of the re-registration eligibility decision (RED) process does justify the proposed numerical standard of 100 ng/L (USEPA 1999). EPA (1999) considered the scud (*Gammarus fasciatus*) to be the most susceptible invertebrate, exhibiting an LC50 of 200 ng/L. EPA uses a deterministic risk quotient (RQ) approach (ratio of exposure concentration to toxicity endpoint) to determine if exposure exceeds their levels of concern (LOC). Any estimated exposure above the LOC would require risk mitigation. For restricted use products the RQ should not exceed 0.1 (i.e., a 10-fold safety or uncertainty factor is applied to the acute toxicity endpoint). Application of the equivalent 10-fold safety factor to the *G. fasciatus* LC50 indicates that any diazinon residues greater than 20 ng/L would exceed EPA's LOC. Thus, the proposed Basin Plan Amendment criterion of 100 ng/L is within a factor of 5 of the EPA's LOC.

For assessment of the risk of chronic toxicity, EPA's LOC is equal to the NOAEC. In the RED for diazinon, EPA noted the most sensitive species was *Daphnia magna*, exhibiting a 21-day NOAEC in a chronic toxicity assay of 170 ng /L. Thus the Basin Plan Amendment target is within a factor of 2 of the benchmark that EPA would employ to trigger risk mitigation.

The Toxic Units Criterion. The Basin Plan seeks to meet the narrative goal of "no acute or chronic toxicity in ambient waters." The narrative essentially contains language that describes the objectives for reaching the goal, namely "All waters shall be maintained free of toxic substances in concentrations that are lethal to or that produce other detrimental responses in aquatic organisms." This objective is also applied to higher levels of ecological organization including detrimental effects on the community ecology. Furthermore, the narrative goal is extended to sediments—"Controllable water quality factors shall not cause a detrimental increase in the concentration of toxic pollutants in sediments or aquatic life." Interestingly, the latter goal differs from the first of no acute or chronic toxicity in ambient waters by stating explicitly "controllable water quality factors" and a success benchmark of no "detrimental increase in the concentration of toxic pollutants". The narrative objective for sediments in urban creeks thus seems to accept that toxic concentrations of contaminants may already exist in the sediments, and that the only action possible is to limit controllable factors. The focus on controllable factors is also applied to population and community level effects. Although such factors are not explicitly stated, by implication they seem to largely center on pesticide use. If controllable factors are but a euphemism for "pesticides", then the Basin Plan seems to be premised on ignoring the plethora of other contaminants related to urban runoff, perhaps because these are uncontrollable.

The Basin Plan proposes to use toxicity testing as a tool to decide whether its actions are meeting the narrative objectives of no ambient water acute or chronic toxicity and no increase in toxic pollutants in sediment. However, the quantitative expression of the toxicity tests via generic Toxic Units (TU) as proposed cannot lead to a valid conclusion that the toxicity is indeed pesticide related.

The proposed toxic units criterion as a numeric target for pesticide residues is problematic as a TMDL for the following reasons. First, the TU expression proposed seems to be an adaptation of the WET procedure for wastewater discharges wherein dilution of the sample is used as a surrogate for "concentration" (recommended procedure in US EPA 1996). The TU amount is calculated as the ratio of 100 to the NOAEC (for acute effects) or NOEC (for chronic effects), where the NOAEC is the percentage dilution of the sample associated with no significant toxicity compared to a control (presumably toxicity in reference water). The use of this TU analysis is generic in that the chemical specific causes of toxicity are unknown. Thus, the TU expression is more qualitative than quantitative. More importantly, however, use of such a generic expression does not address the pesticide use and runoff issue directly because other analytical methods are not explicitly recommended or advised as part of the numeric target. For example, exceedance of the target has been defined in the Plan as a TU greater than 1.0. Without further analytical guidance, it cannot be known whether the toxicity was associated with pesticide residues. If uncertainty about the cause of the toxicity dominates the observation, then how will the Water Quality Control Board know if the TMDL is being met or not?

Because the proposed numeric TMDLs are meant to protect water quality from excessive pesticide contamination and are oriented toward meeting the Basin Plan narrative goal of no toxicity, knowing the cause of toxicity is not trivial if the TMDL is to be used to help implement best management practices (BMPs) for pesticide use and disposition. Research over the last few years has focused more attention on toxicity caused in receiving waters within urban landscapes (Paul and Meyer 2001, Pitt 2002, Schiff and Bay 2003, Greenstein et al. 2004). Bioassessment is recommended to understand the impacts of urban runoff on receiving waters (Pitt 2002), but TIE protocols are necessary to understand specific chemical sources of toxicity. For example, in one study of parking lot wash off, zinc concentration was identified through TIE as the major contributor to toxicity (Greenstein et al. 2004). Thus, without application of TIE methods, how will confounding contaminants in urban runoff be eliminated as a toxicity source? Ironically, only after the application of TIE procedures was diazinon confirmed as a prime cause of *Ceriodaphnia* toxicity in WET tests on ambient water samples from agricultural watersheds and urban streams (e.g., Bailey et al. 2000).

A second problem arises with the generic TU as a numeric standard when non-mortality endpoints are used in the chronic WET tests. Acute WET tests wherein mortality is the sole endpoint have low false negative and false positive rates. WET tests for chronic endpoints like reproduction (i.e., sublethal effects) have been shown in at least one study to have unusually high false positive rates (Moore et al. 2000). Thus, a generic TU numeric standard for chronic toxicity may be prone to false positives, making the progress toward meeting narrative goals inconclusive.

A third problem with the generic TU numeric standard as proposed is uncertainty of how sediment toxicity tests would be conducted. While ambient waters can be diluted with control or reference water, or with receiving water not exhibiting toxicity (USEPA 1996), it is not clear from the Basin Plan Staff Report how sediment toxicity tests would be conducted so that the generic TU could be calculated. A search for sediment dilution procedures in USEPA's "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates" revealed no standardized protocol for diluting sediments collected from the field (USEPA 2000). Sediment dilutions for testing the amphipod *Hyalella azteca* were accomplished using putatively uncontaminated field sediments (i.e., those exhibiting no toxicity) in the watershed where contaminated sediments were collected (Weston et al. 2004). However, no standardized procedure seems to exist for conducting sediment dilution series with field samples. Furthermore, the following question seems germane to interpreting sediment dilution experiments: "Does simply adding uncontaminated sediment (e.g., washed sand) to putatively contaminated sediment constitute a dilution that still maintains a realistic perspective of bioavailability of contaminants that have aged in sediment?"

Proposed Solution to the Generic Toxicity Unit Problems: The Staff Report has been very progressive in attempting to develop a TMDL for the potential toxicity of pesticides that are already substituting for the OP insecticides in urban markets. The Report has shown convincingly that pyrethroid insecticides have become the most commonly used landscape and structural pest control chemicals. Furthermore, studies conducted in agricultural watersheds are now showing that pyrethroid sediment concentrations can exceed the established LC50 for *H. azteca* or *Chironomus tentans* (Weston et al. 2004; Amweg et al. 2005). By analogy to the problems associated with urban uses of diazinon, anticipating potential toxicity from pyrethroids in urban streams is reasonable.

Although the environmental toxicological database on any one pyrethroid insecticide is not as robust as that for commonly used OP insecticides, peer-reviewed information is now available on the LC50 of commonly used pyrethroids in both the ambient water column and in sediment (e.g., Amweg et al. 2005). Furthermore, analytical methods have now been developed that can analyze water and sediments for pyrethroid insecticide residues with detection limits at biologically relevant concentrations (e.g., You 2004). With the availability of specific toxicological endpoints (i.e., lethality based dose-response relationships) and improved analytical methods, the proposed TMDL can now be based on a chemical-specific TU approach.

The specific TU method derives a ratio of the concentration of contaminant in the subject matrix relative to its LC50 (or EC50 for other sublethal effects as information becomes reliable and available). The specific TU approach has already been used in agriculturally influenced waters to hypothesize pyrethroids as causal toxic agents to both water dwelling and sediment inhabiting invertebrates (Weston et al. 2004; Amweg et al.

2005). If multiple pyrethroid residues are found, TUs for individual chemicals can be added assuming additive interactions. However, caution in assuming additivity is necessary because pyrethroids have two distinct modes of biochemical action (MOA) on the axon sodium channel. Nevertheless, pyrethroids can be classified based on specific MOA as Type I (permethrin) and Type II (typically cyano containing pyrethroids, including cypermethrin, cyfluthrin, cyhalothrin, deltamethrin, fenvalerate) (NPTN 1998).

As pyrethroid insecticides receive increased research attention during the eventual agricultural phase-out of OP insecticides, more information will become available to refine the parameters forming the basis of the toxicity TMDL. For example, several published papers question whether the environmental levels of pyrethroid residues in water or sediment have long term ecological relevance owing to the observed recovery potential of aquatic invertebrate communities (as opposed to being toxic to a sensitive invertebrate like *H. azteca*) (e.g., Maund et al. 1998; Schulz and Liess 2001; Schroer et al. 2004). One paper has argued that the route of exposure to pyrethroids is difficult to predict for aquatic invertebrates, and that water concentrations may be more predictive of toxicity to the midge *Chironomus riparius* than the sediment concentrations (Conrad et al. 1999). Nevertheless, the Basin Plan narrative goal may be concisely stated as "no measurable toxicity", and thus the TMDL can be based on acute and chronic testing with the most sensitive invertebrate species regardless of exposure route and the potential for invertebrate population recovery. If in the future, the Basin Plan narrative goal changes to a more realistic ecological perspective (rather than a single organism perspective), the TMDL can be adjusted accordingly.

Total Maximum Daily Load and Allocations:

The development of the TMDL as a specific diazinon concentration or as a toxicity-based standard seems consistent with the flexibility built in to the Clean Water Act regulatory mandates. Furthermore, the toxicity-based TMDL is consistent with the Basin Plan narrative goal of basically "no measurable toxicity". Other California Water Quality Control Boards are using the same approach. As stated above, however, if the TMDL proposed is supposed to address the specific issue of excessive pesticide residues, then the generic TU should be changed to a chemical specific TU. This change is possible at least on an interim basis given recent publications of dose-response estimates for aquatic and sediment dwelling invertebrates exposed to pyrethroids, as well as the use of a specific TU approach for hypothesizing the causes of toxicity in agriculturally influenced waters in California.

Adaptive Implementation:

The adaptive implementation strategy has proposed to calculate monitoring benchmarks in lieu of water quality criteria for discovered pesticide residues that lack criteria. The monitoring benchmark is essentially the LC50 from an acute 4-day ambient water toxicity assay divided by an uncertainty factor (defined as the "benchmark factor"). The uncertainty factor shown in Table 4-x seems to be scaled to the number of genera available with valid dose-response estimates. The scientific validity of such scaling and the subsequent calculation of the monitoring benchmark have been raised as an issue requiring peer review comment.

The use of uncertainty factors to scale measurable dose-response estimates to account for inter- and intra-species sensitivity, as well as age or sex related sensitivity, is routine for science policy. The magnitude of the safety factors is not a scientific judgment but one that weighs the mandates of statutory and regulatory law and the objectives for protection. Thus, the appropriateness of the proposed benchmark factors can only be judged in comparison to other regulatory practices for protection of the environment. Also, the magnitude of the benchmarks can be assessed by asking are they conservative (i.e., protective) enough.

I propose that the EPA ecological risk assessment process as applied to pesticides be examined as a guide to the "validity" of the Basin Plan's proposed monitoring benchmarks. I have often seen authors of publications about pesticide residues in water "complain" that a water quality criteria has not been promulgated for a particular compound, as if we somehow lack a mechanism for judging the biological significance of the residue without the criteria. For all the criticism that the EPA has received regarding it registration review process in light of the mandates for environmental protection, its risk assessment process is quite fine-tuned in being able to tell us the likelihood of adverse effects on single species. For example, as often happens in the risk assessment of the older neurotoxic pesticides that have biochemical modes of action shared by invertebrates and vertebrates (e.g., the anticholinesterase insecticides), the estimated environmental concentrations of residues (EECs) often exceed the EPA's levels of concern (LOCs). The LOCs themselves reflect safety factors that are applied through the use of risk quotients (RQs).

RQs vary by risk scenario and are calculated as the ratio of the EEC to the acute toxicological endpoint (i.e., the LC50) for the most sensitive organism in the aquatic toxicity database. For example, if the EPA deems that a pesticide is to be for restricted use only (an operator needs certification and a license to purchase it), the RQ must be <0.1, meaning that the EEC must be at least 10x lower than the LC50. For protecting endangered species, the RQ must be <0.05, effectively applying a safety factor of 20 to the LC50 to derive a residue concentration that would pose a reasonable certainty of no harm.

The EECs are very conservative, having been derived almost exclusively by transport modeling (using PRZM) and a static water fate model (EXAMS). The applicability of EXAMS to flowing water is extremely dubious, and the vast majority of EECs for different pesticide use scenarios significantly overestimate water residues compared to actual environmental monitoring. Nevertheless, USEPA's RQ approach can be used to determine the likelihood of adverse effects (i.e., acute toxicity) from exposure to a given residue in water. For chronic effects (i.e., reproductive and developmental toxicity), the NOAEC is the benchmark toxicological endpoint and the RQ is set to 1, meaning that residues below the NOAEC for the most sensitive organism do not exceed the LOC.

The magnitudes of the RQs are simply risk management devices, not based on any scientific toxicological principle, but rather tied to science policy's need to implement precaution. Pertinently, the EPA still registers pesticides for certain uses wherein the benchmark RQ has been exceeded. The agency is criticized for such actions, but the critics fail to understand that FIFRA allows environmental risk assessment and management to weigh the risks and benefits of pesticide use. In contrast, the Clean

Water Act mandates for protection of aquatic biota are weighted to consideration of the risks. If the EPA had been mandated to only consider pesticide risks to aquatic organisms and not the corresponding benefits of pesticide use, a number of pesticide registrations may have been cancelled, or at least the product labels (which have force of law) altered to place severe restrictions on use. Evidence for such risk management actions come from examining the effects on certain pesticide registrations of the Food Quality Protection Act, an amendment to FIFRA that changed the risk/benefit assessment for pesticide registration to a risk only assessment when consumer protection (i.e., non-occupational human health) was at stake. Thus, the deterministic risk assessment approach represented by the RQ should be viewed as quite conservative. The argument over the validity of a pesticide registration is really about what the agency should do when the RQ is exceeded. Therefore, given that the EPA RQ approach incorporates safety factors ranging from 2 to 20 fold depending on exposure scenario, I conclude that the range of proposed benchmark factors in the Basin Plan will be just as conservative in ensuring protection of the most sensitive species.

Overarching Questions:

Overall, I conclude that the Staff Report represents a fair assessment of the available scientific monitoring data and putative adverse effects observed in several California watersheds. I also find it commendable that the implementation of the proposed TMDLs will partner with many agencies and all stakeholders are included. My main disagreement is with the use of a generic TU method for other pesticides (namely the pyrethroids) when a chemical specific TU approach can be used given the data published over the last few years.

A widespread and comprehensive education program led by the UC Extension Service and the pest control businesses themselves will be key to successfully meeting the specific TMDLs as well as the narrative goals of the Basin Plan. However, I feel that one piece of data presented in the report was overlooked, yet could be very helpful in focusing the educational efforts. Page 51 of the report stated that 0.25% of the 90 tons (or 450 pounds) of diazinon applied annually throughout the entire Bay Area translocated to surface water. On page 53, survey results suggested that up to 4% of residential pesticide users improperly disposed of waste rinse water and 1.5% improperly disposed of unwanted product itself by dumping in streets, gutters, and drains outside the house. If 50% of the diazinon use was related to "over the counter" purchases, then presumably 45 tons was used by residences rather than professional applicators. Assuming diazinon usage is spread equally among the residents (i.e., each resident uses product at the same rate), then given the incidence of improper disposal, potentially 1350 to 3600 pounds could be placed in areas extremely vulnerable to direct runoff.

One study has shown with different herbicides that sorption is low on asphalt and even lower on concrete (Ramwell 2005). After 6 days of contact with these urban surfaces, 60-90% of the herbicides were washed off with water, suggesting the high potential for mobility. To extrapolate these low sorption potentials to the magnitude of improper diazinon disposal, at 60% washoff, 810-2160 pounds of diazinon could have been carried into street runoff. Thus, the hypothetical magnitude of diazinon runoff resulting from improper waste disposal of unused product and spray tank mix alone could account for the estimated loads of diazinon reaching urban creeks. Assuming this analysis is plausible, I would focus intensely on education efforts that encourage citizens to spray out used material onto soil protected from intense rainfall and dispose of unused products during household hazardous waste disposal events.

References:

Amweg, E. L., D. P. Weston and N. M. Ureda. 2005. Use and toxicity of pyrethroid pesticides in the Central Valley, California, USA. Environmental Toxicology and Chemistry 24 (4):966-972.

Bailey, H. C., L. Deanovic, E. Reyes, T. Kimball, K. Larson, K. Cortright, V. Connor and D. E. Hinton. 2000. Diazinon and chlorpyrifos in urban waterways in northern California, USA. Environ. Toxicol. Chem. 19(1):82-87

Conrad, A. U., R. J. Fleming and M. Crane. 1999. Laboratory and field response of *Chironomus riparius* to a pyrethroid insecticide. Water Research 33(7):1603-1610.

Kuivila KM, Foe CG. 1995. Concentrations, transport and biological effects of dormant spray pesticides in the San Francisco estuary, California. *Environmental Toxicology and Chemistry* 14:1141-50

Moore, T. F., S. P. Canton and M. Grimes. 2000. Investigating the incidence of type I errors for chronic whole effluent toxicity testing using *Ceriodaphnia*. Environmental Toxicology and Chemistry 19 (1):118-122.

Greenstein, D., L. Tiefenthaler and S. Bay. 2004. Toxicity of parking lot runoff after application of simulated rainfall. Arch. Environ. Contam. Toxicol. 47:199-206.

Maund, S. J., M. J. Hamer, J. S. Warinton and T. J. Kedwards. 1998. Aquatic ecotoxicology of the pyrethroid insecticide lambda-cyhalothrin: Considerations for higher-tier aquatic risk assessment. Pesticide Science 54:408-417.

National Pesticide Telecommunications Network. 1998. Pyrethrins and pyrethroids fact sheet. http://npic.orst.edu/factsheets/pyrethrins.pdf

Paul, M. J. and J. L. Meyer. 2001. Streams in the urban landscape. Ann. Rev. Ecol. Syst. 32:333-365.

Pitt, R. 2002. Receiving water impacts associated with urban runoff. In D. Hoffman, B. Rattner, G. A. Burton, Jr., and J. Cairns, Jr. Handbook of Ecotoxicology, 2nd Edition. CRC-Lewis. Boca Raton, FL. pp. 1-37.

Ramwell, C. T. 2005. Herbicide sorption to concrete and asphalt. Pest Management Science 61:144-150.

Schiff, K. and S. Bay. 2003. Impacts of stormwater discharges on the nearshore benthic environment of Santa Monica bay. Marine Environmental Research 56:225-243.

Schroer, A. F. W., J. D. M. Belgers, T. C. M. Brock, A. M. Matser, S. J. Maund and P. J. Van den Brink. 2004. Comparison of laboratory single species and field population-level effects of the pyrethroid insecticide lambda-cyhalothrin on freshwater invertebrates. Arch. Environ. Contam. Toxicol. 46:324-335.

Schulz, R. and M. Liess. 2001. Acute and chronic effects of particle-associated fenvalerate on stream macroinvertebrates: A runoff simulation study using outdoor microcosms. Arch. Environ. Contam. Toxicol. 40 481-488.

Siepmann S, Finlayson BJ. 2000. Water quality criteria for diazinon and chlorpyrifos. *California Department of Fish & Game, Office of Spill Prevention and Response, Administrative Report 00-3.*

USEPA. 1996. Regions 9 & 10 Guidance for Implementing Whole Effluent Toxicity Testing Programs. pp. 3-14 & 3-15.

USEPA. 1999. EFED RED Chapter for Diazinon. (Document available through links at http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg)

USEPA. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-99/064, March 2000 2nd Edition 192 pp.

Werner I, Deanovic LA, Connor V, de Vlaming V, Bailey HC, Hinton DE. 2000. Insecticide-caused toxicity to Ceriodaphnia dubia (Cladocera) in the Sacramento-San Joaquin River Delta, California, USA. *Environ. Toxicol. Chem.* 19: 215-27

Weston, D. P., J. You and M. J. Lydy. 2004. Distribution and toxicity of sediment-associated pesticides in agriculture-dominated water bodies of california's central valley. Environ. Sci. Technol. 38:2752-2759.

You, J., D. P. Weston and M. J. Lydy. 2004. A sonication extraction method for the analysis of pyrethroid, organophosphate, and organochlorine pesticides from sediment by gas chromatography with electron-capture detection. Arch. Environ. Contam. Toxicol. 47 141-147.